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Role of timing of exposure to pets and dampness or mold on asthma and sensitization in adolescence

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Authors' contributions

BB and HAS were responsible for the conception and design of the PIAMA study. GHK, AHW, HAS, and UG secured funding for the present study. EM and UG designed the study and had full access to the data. EM carried out the statistical analyses and wrote the initial draft of the manuscript. All authors (i) provided substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of the data for the work, (ii) revised the manuscript critically for important intellectual content, and (iii) approved the final version for submission.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from authorized institutional review boards. Children's parents or legal guardians and children themselves provided written informed consent

Availability of data and material

The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Gerard Koppelman received grants from Netherlands Lung Foundation, grants from Ubbo Emmius Foundation, grants from TEVA the Netherlands, grants from Stichting Astma Bestrijding, outside the submitted work. Ulrike Gehring reports receiving grants from the Dutch Lung foundation during the conduct of this study. All other authors declare no potential conflicts of interest.

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Abstract

Background: Pet and dampness or mold exposure are considered risk factors for asthma and sensitization. It is unclear whether timing of exposure to these factors is differentially associated with asthma risk and sensitization in adolescence.

Objective: We investigated the role of timing of pet and dampness or mold exposure in asthma and sensitization in adolescence. Understanding this role is essential to build targeted prevention strategies.

Methods: We used data from 1871 participants of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort. Residential exposure to pets, dampness or mold was assessed by repeated parental questionnaires. We used asthma data from the 17-year questionnaire and sensitization data from the 16-year medical examination. We characterized timing using longitudinal exposure patterns from pregnancy till age 17 using longitudinal latent class growth modelling. We used logistic regression models to analyze associations of exposure patterns with asthma at age 17 and sensitization at age 16.

Results: For none of the time windows, exposure to pets and dampness or mold was associated with asthma at age 17, but a lower sensitization risk at age 16 was suggested, e.g. the odds ratio (95% confidence interval) for sensitization was 0.63 (0.35 to 1.11) and 0.69 (0.44 to 1.08) for early life and persistently high pet exposure ,respectively, compared to very low exposure. An inverse association was also suggested for sensitization and moderate early childhood dampness or mold exposure [0.71 (0.42 to 1.19)].

Conclusion and clinical relevance: Different timing of pet and dampness or mold exposure was not associated with asthma, but lower risk of sensitization in adolescence was suggested, which could be partly attributable to reversed causation. Current findings are not sufficient to

recommend pet avoidance to prevent allergic disease. More prospective studies are needed to obtain insights that can be used in clinical practice.

Keywords: Asthma, Sensitization, Adolescence, Dampness and mold, Pets**.**

Introduction

The impact of exposure to pets and dampness or mold on asthma and sensitization in children has been shown previously,[1-5] and it has been suggested that environmental exposures during important windows of immune development play a role in the risk of subsequent allergic disease development. [7, 8]

Systematic reviews of the association between pet exposure and asthma present inconsistent evidence. While some studies suggest that pet exposure is associated with a higher risk of asthma,[2, 9-11] others suggest a lower asthma risk in exposed individuals. [7, 12, 13] A pooled analysis of 11 European cohorts found no association between keeping furry pets early in life and asthma in children aged 6-10 years.[14] Another study investigated associations of pet exposure during different periods of childhood with asthma and also found no association of early, past and current pet exposure with asthma in schoolchildren.[15] Sensitization to inhalant allergens is considered an important risk factor for the development of asthma [16] and exposure to pets in early life has been consistently associated with lower risk of sensitization during childhood,[1, 14, 17] but it is unknown whether this inverse relationship persists into adolescence and whether exposure during other periods is relevant.

Reviews of the epidemiological evidence for respiratory and allergic health effects of dampness or mold exposure have consistently suggested higher risks of asthma in exposed children.[3, 4, 18] However, limited evidence exists on the associations of dampness or mold exposure with sensitization though a higher risk of sensitization has been observed in exposed children.[19] Few studies have assessed associations of dampness or mold exposure with asthma or sensitization beyond childhood into adolescence. A study that addressed this gap [20] reported a higher risk of asthma up to 16 years in relation to exposure to dampness or mold during infancy, but no association with sensitization was observed.

Existing literature on associations of pet or dampness or mold exposure with asthma and sensitization has either focused on exposure in early life and/or asthma and sensitization in early life and childhood. Therefore, not much evidence exists on the associations of lifecourse exposure and the relevance of timing of exposure during different periods until adolescence. Investigating the timing of exposure is essential as exposure during different time periods in the lifecourse may differentially affect development of asthma and sensitization and may thus have consequences for the timing and type of preventive measures. [16]

It is possible that the effect of pet or dampness or mold exposure may differ in different phases of the development of the immune system. The perinatal time window is crucial as the infant's immune system is vulnerable, and the development of the immune response is ongoing. [21] And in childhood, there is a shift from Th2 cells dominated immune response to Th1 dominated responses.[22] As such, as age advances, the immune system also undergoes profound remodelling and decline, which may have impact on lifecourse health outcomes.[23] We hypothesize that exposure to pets and dampness or mold during different stages of childhood would differentially affect asthma and sensitization prevalence in adolescence. We therefore used longitudinal patterns of exposure from pregnancy to adolescence, to investigate the relevance of timing of pet and dampness or mold exposure for the prevalence of asthma at age 17 and sensitization at age 16.

Methods

Study design and population

We used data from the Dutch PIAMA birth cohort that has been described in detail elsewhere.[24] The cohort recruited pregnant women between 1996-97 in the Northern,

Central and Western regions of the Netherlands. Information on lifestyle, health, and environmental exposure characteristics were collected using parental questionnaires that were administered during pregnancy, at 3 months, annually until age 8, and then at ages 11, 14, 16 (for the subgroup that participated in the medical examination) and 17. The study population consists of all participants with data on sensitization at age 16 and/or asthma at age 17, and data on exposure to pets or dampness or mold from at least one follow-up (N=1871). The PIAMA study was approved by the institutional review boards of participating institutes and written informed consent was obtained from parents or legal guardians of all participants.

Exposure assessment

Exposure was assessed from pregnancy (pets) and 3 months (dampness or mold) until age 17.

Pet exposure

The question '*Do you keep a dog/cat/rodent indoors?*' (*yes, no)* was used to assess exposure to furry pets. The question was asked separately for each pet.

Dampness or mold

The question *'Have you seen any moisture stains or mold on the ceiling or walls in the last 12 months?'* (*yes, no)* was used to assess dampness or mold exposure. Assessment was restricted to presence of dampness or mold in the living room and the child's bedroom because this is where participants are expected to spend most of their time.

Longitudinal patterns of exposure

We characterized time-varying binary exposures into longitudinal patterns using latent class growth modeling procedure (LCGM, TRAJ in SAS 9.4, Cary, USA) as in previous analyses.[25]

We used this approach unlike using distinct time windows (e.g. prenatal, preschool, primary, secondary school time windows) because it allocates individuals based on probability of exposure rather than subjective definitive assignment of individuals into classes and it can handle missing data while using all available data.[26, 27] In addition, it is a data driven procedure that displays subpopulations of individuals with different patterns of lifecourse exposure indicating exposure during specific phases of follow-up. All participants with available data on pet or dampness or mold exposure from at least one of the repeated questionnaire surveys were included in the latent class modelling procedure, i.e. all available data was used. Table S1 presents the frequencies of questionnaire surveys with missing values for the different exposures. We used questionnaires from 13 waves of follow-up. Only 6% and 9% of the study population had missing data of more than 2 waves for pet and dampness or mold exposure respectively. The different patterns obtained in the procedure were used as exposure variables in statistical analyses of exposure-health relationships.

Outcomes

Asthma at age 17 was defined by positive answers to at least two out of the following three questions as described by the MeDALL protocol [28]: doctor diagnosed asthma ever, wheezing in the past 12 months, and prescription of asthma medication in the past 12 months.

Sensitization at age 16 was assessed in a subgroup of participants that participated in the medical examination (N=682) and defined as a specific IgE level ≥ 0.35 IU/mL for at least one of the following allergens: house dust mite (HDM, *Dermatophagoides pteronyssinus*), cat allergen, birch, and cocksfoot (*Dactylis glomerata)*. Specific IgE levels were measured with a Radioallergosorbent test-like method (Sanquin Laboratories, Amsterdam, The Netherlands).

0.35 IU/mL was chosen as the primary cut-off point because it is commonly used in epidemiological research and clinical practice.

Confounders

The following factors were considered as potential confounders: sex, parental education (maximum of maternal and paternal education, low/medium/high), maternal and paternal allergy (defined as positive if the father and/or mother ever had asthma, were allergic to house dust, house dust mite or pets, or had hay fever), breastfeeding at 12 weeks (yes/no), parental country of birth (Netherlands, yes/no), maternal smoking during pregnancy (yes/no), secondhand smoke (SHS) exposure in the child's home at 1 year (yes/no), active smoking (at 17 years, yes/no), gas cooking at 3 months (yes/no), the presence of older siblings (yes/no), respiratory infections (serious cold or flu, infection of the throat, otitis media, sinusitis, bronchitis or pneumonia) in the first 4 years of life and antibiotic use in the first 4 years of life (never, at least once*)*. In addition, we adjusted for furry pets in the home at 1 year (yes/no) in analyses with dampness or mold exposure and for dampness or mold in the home at 1 year (yes/no) in models with pet exposure.

Statistical analysis

We used logistic regression to assess crude and adjusted associations of different patterns of exposure with asthma at age 17 and sensitization at age 16. All models were adjusted for the previously mentioned potential confounders. Observations were weighted by posterior probabilities produced by the latent class modeling procedure to account for uncertainties in the allocation of longitudinal exposure patterns.[29]

A number of sensitivity analyses were performed. We investigated associations of the exposures of interest with allergic sensitization to specific inhalant allergens i.e. cat, house dust mite, birch and cocksfoot allergens to explore how different timing of exposure may be associated with sensitization to the specific inhalant allergens. We performed stratified analyses by parental allergy as predisposition to asthma and allergy may influence the risk of disease and (avoidance of) exposure to pets.[30] Consequently, to investigate if pet avoidance behaviour distorted associations of pet exposure with asthma and sensitization, we repeated pet exposure analyses after excluding parents who reported getting rid of a pet at any point during follow up due to an allergy of a family member (N=246). We also analysed associations of exposure to different pets (cats, dogs, rodents) with asthma and sensitization separately as different pets have been suggested to have different effects on asthma/sensitization.[21] To assess if weighting observations by posterior probabilities influenced our results, we repeated the main analyses without using weights, i.e. by allocating subjects to the exposure trajectory with the highest posterior probability. We also assessed associations of the exposures of interest with sensitization using a higher IgE cut off of 0.7 IU/mL to investigate the influence of a different cut off point. Moreover, we investigated associations of pet and dampness or mold exposure with mono- and polysensitization (i.e. sensitization to only one allergen and more than one allergen) versus no sensitization using multinomial logistic regression.

Results

Table 1 shows characteristics of the study population. Twenty-nine percent of the participants had an allergic mother and 31% had an allergic father. Thirteen percent had mothers who smoked during pregnancy and 22% were exposed to SHS at home; 42% were exposed to pets and 8% were exposed to dampness or mold in the first year of life. Five percent of the

169 population was asthmatic at age 17 and 48% was sensitized to at least one of the inhalant
170 allergens tested at age 16. Participants in the study population were more often breastfed for
171 more than 12 weeks, less often exposed to SHS at home at 1 year and to maternal smoking
172 during pregnancy, and more often had highly educated parents than the excluded population
173 (Table S2).

174 Figure 1 shows the longitudinal patterns of pet and dampness or mold exposure from
175 pregnancy (pets) or 3 months (dampness or mold) to 17 years. The mean posterior
176 probabilities per pattern ranged from 0.90 - 0.97 for pet exposure and 0.75 – 0.92 for
177 dampness or mold exposure indicating reliable classification of membership (Table S3). Five
178 distinct patterns of pet exposure reflecting timing of exposure were identified as follows: very
179 low (28%) indicating very low probability of exposure throughout follow-up, early life
180 (11.1%) indicating high probability of exposure in early life, mid-childhood (14%) indicating
181 high probability of exposure in mid-childhood, late childhood (14%) indicating high
182 probability of exposure later in childhood and persistently high exposure (31%) showing high
183 probability of high exposure during the entire follow up. We identified three patterns of
184 dampness or mold exposure: very low (79%) characterized by a very low probability of
185 exposure throughout follow-up, moderate early childhood (11%) with only moderate
186 probability of exposure in early life and moderate late childhood (9%) with moderate
187 probability of exposure in late childhood. Distributions of study characteristics among
188 patterns of exposure are presented in Tables S4 and S5. The very low pet exposure pattern
189 was characterized by more children with allergic and highly educated parents and less
190 participants exposed to maternal smoking during pregnancy and SHS in the home. The
191 persistently high pattern was characterized by less participants with allergic parents and less
192 highly educated parents and more participants exposed to maternal smoking during

pregnancy and SHS in the home. Study characteristics were evenly distributed between dampness or mold exposure patterns.

Figure 2 shows adjusted associations of longitudinal patterns of exposure with asthma at age 17 and sensitization at age 16. Crude and adjusted odds ratios were generally similar (Table S6). We did not observe consistent associations of any of the pet exposure patterns with the risk of asthma at age 17 compared to very low exposure, but a higher risk of asthma was suggested for early life pet exposure [OR (95% CI) 1.66 (0.86 to 3.19)]. All patterns of pet exposure, however, tended to be consistently associated with a lower risk of sensitization at age 16 [0.63 (0.35 to 1.11)] for early life pet exposure and [0.69 (0.44 to 1.08)] for persistently high pet exposure as compared to very low exposure. No significant associations with patterns of dampness or mold exposure were observed for asthma, but a tendency of a lower risk of sensitization was also observed (Table S6).

Sensitivity analyses

When we assessed associations of pet and dampness or mold exposure with allergic sensitization to specific allergens, early life, late childhood and persistently high pet exposure was associated with lower risk of sensitization to birch, house dust mite and cocksfoot allergens at age 16 (Table S7). Dampness or mold exposure was also significantly associated with a lower risk of cat [0.15 (0.03 to 0.64) for moderate late childhood exposure] and house dust mite allergen sensitization [0.55 (0.32 to 0.96) for moderate early childhood exposure] (Table S7).

Stratification by parental allergy showed similar associations as in the main analyses and there were no differences in associations between children born to allergic and non-allergic

parents (Table S8). Excluding participants whose parents reported getting rid of pets at any point during follow-up due to an allergy of a family member did not change results though higher risk of asthma was suggested. (Table S9).

Non- significant lower risks of sensitization were consistently observed with cat and dog exposure, but not with exposure to rodents in analyses with patterns of exposure to separate pets. We did not observe any associations with exposure to specific pets for asthma. (Figure S2 and Figure S3). When we repeated the main analyses without weighting by posterior probabilities, the weighted and unweighted analyses produced similar estimates (Table S10).

Likewise estimates were similar in sensitivity analyses using a higher cut off value (0.7 IU/mL) for sensitization except for a significant inverse association between early life pet exposure and sensitization to at least one allergen and stronger associations of early life pet exposure with sensitization to specific allergens (Table S11*).* A lower risk of both, polysensitization and monosensitization was suggested for all time windows of pet and dampness or mold exposure (Table S12), but few associations were statistically significant as numbers became small.

Discussion

In our prospective birth cohort, we did not find associations of different timing of pet and dampness or mold exposure from pregnancy/birth till adolescence, with asthma at age 17 compared to very low exposure, but any pet and dampness or mold exposure during the lifecourse tended to be consistently associated with a lower risk of sensitization at age 16.

Timing of pet exposure

Studies have shown both higher [2, 9, 10, 31, 32] and lower risks [1, 12, 13, 17] of asthma and sensitization among those exposed to pets. A pooled analysis of 11 European birth cohorts including our own did not find an association between pet exposure in the first two years and asthma at ages 6-10 but observed a lower risk of sensitization.[14] To our knowledge no other study has investigated the relevance of the timing of pet exposure in associations with asthma and sensitization in adolescence. We did not observe significant associations of any time window of pet exposure with asthma in adolescence but risk of asthma was suggested for early life pet exposure partly in line with studies that have reported higher risk of asthma in relation to early life pet exposure. [31] Consistent inverse associations were suggested for sensitization when different timing patterns were compared to low exposure.

Separate analyses of the associations of allergic sensitization to specific allergens with pet exposure, suggested that compared to very low exposure, early life, late childhood and persistently high pet exposure may be associated with lower risks of sensitization to house dust mite, cocksfoot and birch allergen. Results of a previous analysis within our cohort showed inverse associations of pet exposure with sensitization and null associations with asthma at age 8.[33] Our current findings extend the exposure period until adolescence and taken together, our set of findings suggests that in our cohort, pet exposure from birth until adolescence is not associated with asthma in adolescence and that the inverse associations with sensitization persist into adolescence.

An important issue regarding the current findings concerns (reverse) causality. Children with allergic parents were over-represented among participants with a very low probability of exposure during the entire follow-up, suggesting that avoidance behavior may at least partly explain the suggested inverse association. Children born to allergic parents are predisposed to develop asthma or become sensitized, and allergic parents are more likely to avoid keeping pets in the home. Consequently, such avoidance behavior can be a source of bias in estimating

the associations between pet exposure and allergic outcomes. We investigated the impact of avoidance of pets by allergic parents in stratified analyses by parental allergy and by excluding participants whose parents got rid of pets during follow-up because of allergies of a family member. We found similar associations for children of allergic and non-allergic parents. However in analyses where we excluded participants whose parents got rid of pets during follow-up a higher risk of asthma was suggested. Therefore, while our results indicate that it is unlikely that the suggested inverse associations are driven by avoidance of pets by allergic parents, reverse causation cannot be completely ruled out.

The suggested lower risk of sensitization to at least one allergen tested and allergic sensitization to specific inhalant allergens observed in our study is in line with the findings of another study that reported inverse associations of early life pet exposure with total IgE levels among allergic individuals up to 18 years old,[34] and is in line with the so-called hygiene hypothesis. The hygiene hypothesis links a favourable maturation of the immune system with exposure to microbes in childhood [35, 36] and is supported by studies reporting lower risks of sensitization in children growing up on farms with farm animals as compared to children growing up without farm animals.[37, 38] The associations of proximity to farm animals are however less consistent with asthma.[39] The mechanisms underlying the inverse association are not clear. For example, it has been suggested that exposure to cat allergens may reduce the risk of asthma and sensitization due to a modified Th2 response characterized by production of IgG4 antibodies produced in response to cat allergen exposure.[40, 41] Alternatively, the presence of endotoxins, which is associated with the presence of pets in the home [42-44] may explain the suggested lower risks of allergic sensitization among those exposed to pets. Endotoxin exposure early in life might promote Th1 cell differentiation, which might reduce the risk of any allergen sensitization.[45, 46]

Timing of dampness or mold exposure

Higher risks of asthma and allergic sensitization in relation to dampness or mold exposure have been reported in several studies,[3, 4, 18, 47, 48] while null associations have been reported in others.[31, 49] A meta-analysis of eight European birth cohorts including our own, reported a positive association of early exposure to visible mold and/or dampness with asthma, but not with sensitization against inhalant allergens at early school age.[48] Few studies have been able to assess association between dampness and/or mold and asthma or sensitization beyond childhood into adolescence. We found no evidence of an association between different timing of exposure to dampness or mold and asthma in the current study, but a tendency towards a lower risk of sensitization in adolescence among participants moderately exposed in early life and late childhood was suggested. A study like ours investigated association of dampness or mold exposure in early life with asthma and sensitization in adolescence and reported a higher risk of asthma up to age 16, but no associations with sensitization in contrast with our findings. [20] However, that study only investigated early life exposure and not different timing of exposure. The lower risk of sensitization in relation to dampness or mold exposure suggested in our study may be explained by presence of mold derived agents such as β (1,3)-glucans, which may be associated with a lower risk of sensitization to inhalant allergens. [18, 50] While we did not observe positive associations with asthma, multiple reviews have suggested that dampness or mold exposure is associated with a higher risk of asthma. [4, 47] Biological mechanisms including inflammatory and immunosuppressive responses to exposure to mold spores, and components of microbial agents have been suggested [3] though the wide variety of health effects associated with dampness or mold cannot be explained by a single mechanism. [4]

Strengths and limitations

An important strength of our study is the availability of detailed information about exposure from birth till age 17. This allowed us to characterize longitudinal patterns of exposure over time and therefore investigate timing of exposure in relation to asthma and sensitization in adolescence. Few other studies so far have (included) exposure data beyond childhood. The prospective design of our study implied small liability of recall bias. We were also able to investigate reverse causation due to allergy of family members which is a common problem in studies assessing associations of pet exposure with allergic outcomes.

We acknowledge several limitations of our study. We relied on parental reports as proxies of pet and dampness or mold exposure assessment which can introduce misclassification of exposure as parents may underreport exposure leading to under estimation of exposure estimates. However, we expect that this misclassification is likely non-differential. Collecting dust samples from homes and analyzing these samples e.g. for their contents of allergens, endotoxin and other biocontaminants could be a more objective assessment for pet exposure, but it is costly for a large study like ours and reflects exposure at one or more specific points in time rather than lifecourse exposure. Visible mold reports, however, have been reported to be highly correlated with airborne concentrations of fungal spores [51] suggesting self-reports of dampness or mold are a good exposure indicator. We were also unable to include factors which might alter some of our observed associations, e.g. frequency and type of contact between children and pets outside the child's home. We only assessed residential indoor exposure and it may be possible that the indoor environment is less important in the etiology of asthma in adolescence than it is in childhood [31] with children spending less time in the home as they grow older. However, exposure outside the home was beyond the scope of this study. A potential limitation of the latent trajectory modelling procedure is that classification of individuals depends on the study population and therefore not exactly the same set of classes may be replicated in a different study population with different exposure

patterns. We are not aware of other studies that used this method to classify exposure, but this method has been used for classification of trajectories of atopic dermatitis and wheeze and similar trajectories have been found in different cohorts,[52, 53] which suggests that replication may be possible in comparable settings. Another limitation is that asthma status was assessed from questionnaires and not based on lung function tests. However, the questionnaire-based outcome is used in large birth cohort studies [54, 55]and it offers data for many subjects, while lung function measurements are more costly and therefore often not feasible for all participants.

There were more highly educated parents, fewer mothers who smoked during pregnancy and fewer participants breastfed and exposed to secondhand smoke in the study population than in the excluded PIAMA population. This may affect generalizability, given that highly educated parents may be less likely to keep pets and less likely to smoke. However, we assume that the associations of potential predictors of pet and dampness or mold exposure with asthma and sensitization, would not be different in the general population with comparable levels of pet ownership. Generalizability may be limited beyond the Dutch population with different levels of pet ownership because varying prevalence of asthma and sensitization, pet ownership rates across countries and varying cultural/ lifestyle differences may present different associations.[56] For example, the higher/lower the frequency of pet ownership in a given community the higher/lower the degree of allergen dispersal in pet-free homes.[57]

In conclusion, we found no evidence of a difference in risk of asthma in adolescence with different timing of pet or dampness or mold exposure as compared to those with very low exposure. A lower risk of sensitization was suggested for all time windows of pet and dampness or mold exposure, but may partly be attributable to reversed causation. While this study adds to the evidence that the risk of sensitization in adolescence might be lower among

364 those with exposure to pets, current evidence from the literature is not strong enough to
365 recommend parents of (young) children to acquire pets to reduce risk of developing allergies.
366 On the other hand, there seems to be no evidence for couples to get rid of pets when expecting
367 a child. More prospective studies establishing a temporal link between pet exposure and
368 asthma and sensitization in adolescence are needed get more insights into this relationship
369 that can then be used in clinical practice when advising parents about acquiring pets in the
370 home.
371

Competing interests

Gerard Koppelman received grants from Netherlands Lung Foundation, grants from Ubbo Emmius Foundation, grants from TEVA the Netherlands, grants from Stichting Astma Bestrijding, outside the submitted work. Ulrike Gehring reports receiving grants from the Dutch Lung foundation during the conduct of this study. All other authors declare no potential conflicts of interest.

372

373 **Table 1.** Study population characteristics

	Study population (N=1871)	
	N/n	(%)
Covariates		
Parental allergy ¶		
Allergic mother	551/1871	29.4
Allergic father	585/1871	31.2
Boys	926/1871	49.4
Presence of pets at 1 year	792/1862	42.5
Presence of mold at 1 year	152/1827	8.3
Breastfeeding >12 weeks	1044/1861	56.1
Gas cooking at 3 months	1550/1864	83.1
Maternal smoking during pregnancy	245/1857	13.2
SHS exposure in the home at 1 year	411/1866	22.0
Respiratory infection in the first 4 years of life ¥	1469/1843	79.7
Antibiotics use in the first 4 years of life	989/1854	53.3
Parental education		
Low	177/1865	9.4
Medium	600/1865	32.2
High	1088/1865	58.4
Active smokers at 17 years	155/1871	8.3
Older siblings at birth	919/1871	49.1
Parental country of birth (Netherlands)	1763/1845	95.6
Health outcomes		
Asthma at age 17	96/1871	5.1
Allergic sensitization at age 16, IgE ≥ 0.35 IU/L		
Sensitization to at least one allergen	328/682	48.1
Sensitization to cat	97/682	14.2
Sensitization to house dust mite (*D. pteronyssinus*)	260/682	38.1
Sensitization to birch	114/682	16.7
Sensitization cocksfoot (*Dactylis glomerata)*	193/682	28.3
Mono-sensitization ‡	126/682	18.5
Poly-sensitization β	202/682	29.6
Allergic sensitization at age 16, IgE ≥ 0.70 IU/L		

Sensitization to at least one allergen	296/682	43.4
Sensitization to cat	79/682	11.6
Sensitization to house dust mite (D. pteronyssinus)	230/682	33.7
Sensitization to birch	96/682	14.1
Sensitization cocksfoot (Dactylis glomerata)	175/682	25.6

¶ ever had asthma, allergic to house dust, house dust mite or pets, or had hay fever

¥ - Respiratory and/or throat-, nose,- ear infections, such as cold, infection of the throat, infection of the middle ear, sinusitis, bronchitis or pneumonia

‡ sensitization to only one allergen, specific IgE level ≥ 0.35 IU/mL

β sensitization to more than one allergen , specific IgE level ≥ 0.35 IU/mL

REFERENCES

1. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288: 963-972.
2. Apelberg BJ, Aoki Y, Jaakkola JJ. Systematic review: Exposure to pets and risk of asthma and asthma-like symptoms. *J Allergy Clin Immunol* 2001; 107: 455-460.
3. Quansah R, Jaakkola MS, Hugg TT*, et al.* Residential dampness and molds and the risk of developing asthma: a systematic review and meta-analysis. *PLoS One* 2012; 7: e47526.
4. Mendell MJ, Mirer AG, Cheung K*, et al.* Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. *Environ Health Perspect* 2011; 119: 748-756.
5. Dick S, Friend A, Dynes K*, et al.* A systematic review of associations between environmental exposures and development of asthma in children aged up to 9 years. *BMJ Open* 2014; 4: e006554.
6. WHO. WHO guidelines for indoor air quality: dampness and mould; 2009.
7. Lodge CJ, Allen KJ, Lowe AJ*, et al.* Perinatal cat and dog exposure and the risk of asthma and allergy in the urban environment: a systematic review of longitudinal studies. *Clin Dev Immunol* 2012; 2012: 176484.
8. Holt PG, Sly PD. Non-atopic intrinsic asthma and the 'family tree' of chronic respiratory disease syndromes. *Clin Exp Allergy* 2009; 39: 807-811.
9. Brunekreef B, Von Mutius E, Wong G*, et al.* Exposure to cats and dogs, and symptoms of asthma, rhinoconjunctivitis, and eczema. *Epidemiology* 2012; 23: 742-750.
10. Luo S, Sun Y, Hou J*, et al.* Pet keeping in childhood and asthma and allergy among children in Tianjin area, China. *PLoS One* 2018; 13: e0197274.
11. Lombardi E, Simoni M, La Grutta S*, et al.* Effects of pet exposure in the first year of life on respiratory and allergic symptoms in 7-yr-old children. The SIDRIA-2 study. *Pediatr Allergy Immunol* 2010; 21: 268-276.
12. Perzanowski MS, Ronmark E, Platts-Mills TA*, et al.* Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002; 166: 696-702.
13. Waser M, von Mutius E, Riedler J*, et al.* Exposure to pets, and the association with hay fever, asthma, and atopic sensitization in rural children. *Allergy* 2005; 60: 177-184.
14. Lodrup Carlsen KC, Roll S, Carlsen KH*, et al.* Does pet ownership in infancy lead to asthma or allergy at school age? Pooled analysis of individual participant data from 11 European birth cohorts. *PLoS One* 2012; 7: e43214.
15. Medjo B, Atanaskovic-Markovic M, Nikolic D*, et al.* Association between pet-keeping and asthma in school children. *Pediatr Int* 2013; 55: 133-137.
16. Donovan CE, Finn PW. Immune mechanisms of childhood asthma. *Thorax* 1999; 54: 938-946.
17. Hesselmar B, Aberg N, Aberg B*, et al.* Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999; 29: 611-617.
18. Tischer C, Chen CM, Heinrich J. Association between domestic mould and mould components, and asthma and allergy in children: a systematic review. *Eur Respir J* 2011; 38: 812-824.
19. Weinmayr G, Gehring U, Genuneit J*, et al.* Dampness and moulds in relation to respiratory and allergic symptoms in children: results from Phase Two of the International Study of Asthma and Allergies in Childhood (ISAAC Phase Two). *Clin Exp Allergy* 2013; 43: 762-774.
20. Thacher JD, Gruzieva O, Pershagen G*, et al.* Mold and dampness exposure and allergic outcomes from birth to adolescence: data from the BAMSE cohort. *Allergy* 2017; 72: 967-974.

21. Chen CM, Tischer C, Schnappinger M*, et al.* The role of cats and dogs in asthma and allergy--a systematic review. *Int J Hyg Environ Health* 2010; 213: 1-31.
22. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000; 55: 688-697.
23. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* 2015; 282: 20143085.
24. Wijga AH, Kerkhof M, Gehring U*, et al.* Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *Int J Epidemiol* 2014; 43: 527-535.
25. Milanzi EB, Brunekreef B, Koppelman GH*, et al.* Lifetime secondhand smoke exposure and childhood and adolescent asthma: findings from the PIAMA cohort. *Environ Health* 2017; 16: 14.
26. Nagin DS. Group-based trajectory modeling: an overview. *Ann Nutr Metab* 2014; 65: 205-210.
27. Niyonkuru C, Wagner AK, Ozawa H*, et al.* Group-based trajectory analysis applications for prognostic biomarker model development in severe TBI: a practical example. *J Neurotrauma* 2013; 30: 938-945.
28. Pinart M, Benet M, Annesi-Maesano I*, et al.* Comorbidity of eczema, rhinitis, and asthma in IgE-sensitised and non-IgE-sensitised children in MeDALL: a population-based cohort study. *Lancet Respir Med* 2014; 2: 131-140.
29. Bakk Z, Vermunt JK. Robustness of Stepwise Latent Class Modeling With Continuous Distal Outcomes. *Structural Equation Modeling: A Multidisciplinary Journal* 2015; 23: 20-31.
30. Almqvist C, Egmar AC, Hedlin G*, et al.* Direct and indirect exposure to pets - risk of sensitization and asthma at 4 years in a birth cohort. *Clin Exp Allergy* 2003; 33: 1190-1197.
31. McConnell R, Berhane K, Gilliland F*, et al.* Indoor risk factors for asthma in a prospective study of adolescents. *Epidemiology* 2002; 13: 288-295.
32. Collin SM, Granell R, Westgarth C*, et al.* Pet ownership is associated with increased risk of non-atopic asthma and reduced risk of atopy in childhood: findings from a UK birth cohort. *Clin Exp Allergy* 2015; 45: 200-210.
33. Kerkhof M, Wijga AH, Brunekreef B*, et al.* Effects of pets on asthma development up to 8 years of age: the PIAMA study. *Allergy* 2009; 64: 1202-1208.
34. Wegienka G, Johnson CC, Havstad S*, et al.* Indoor pet exposure and the outcomes of total IgE and sensitization at age 18 years. *J Allergy Clin Immunol* 2010; 126: 274-279, 279 e271-275.
35. Daley D. The evolution of the hygiene hypothesis: the role of early-life exposures to viruses and microbes and their relationship to asthma and allergic diseases. *Curr Opin Allergy Clin Immunol* 2014; 14: 390-396.
36. Fall T, Lundholm C, Ortqvist AK*, et al.* Early Exposure to Dogs and Farm Animals and the Risk of Childhood Asthma. *JAMA Pediatr* 2015; 169: e153219.
37. Braun-Fahrlander C, Gassner M, Grize L*, et al.* Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin Exp Allergy* 1999; 29: 28-34.
38. Riedler J, Eder W, Oberfeld G*, et al.* Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000; 30: 194-200.
39. von Mutius E. Asthma and allergies in rural areas of Europe. *Proc Am Thorac Soc* 2007; 4: 212-216.
40. Platts-Mills T, Vaughan J, Squillace S*, et al.* Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357: 752-756.

41. Hesselmar B, Aberg B, Eriksson B*, et al.* High-dose exposure to cat is associated with clinical tolerance--a modified Th2 immune response? *Clin Exp Allergy* 2003; 33: 1681-1685.
42. Heinrich J, Gehring U, Douwes J*, et al.* Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy* 2001; 31: 1839-1845.
43. Waser M, Schierl R, von Mutius E*, et al.* Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. *Clin Exp Allergy* 2004; 34: 389-397.
44. Gehring U, Bischof W, Borte M*, et al.* Levels and predictors of endotoxin in mattress dust samples from East and West German homes. *Indoor Air* 2004; 14: 284-292.
45. Abraham JH, Finn PW, Milton DK*, et al.* Infant home endotoxin is associated with reduced allergen-stimulated lymphocyte proliferation and IL-13 production in childhood. *J Allergy Clin Immunol* 2005; 116: 431-437.
46. Gereda JE, Leung DY, Thatayatikom A*, et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355: 1680-1683.
47. Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air* 2007; 17: 284-296.
48. Tischer CG, Hohmann C, Thiering E*, et al.* Meta-analysis of mould and dampness exposure on asthma and allergy in eight European birth cohorts: an ENRIECO initiative. *Allergy* 2011; 66: 1570-1579.
49. Tischer C, Gehring U, Chen CM*, et al.* Respiratory health in children, and indoor exposure to (1,3)-beta-D-glucan, EPS mould components and endotoxin. *Eur Respir J* 2011; 37: 1050-1059.
50. Gehring U, Heinrich J, Hoek G*, et al.* Bacteria and mould components in house dust and children's allergic sensitisation. *Eur Respir J* 2007; 29: 1144-1153.
51. Haas D, Habib J, Galler H*, et al.* Assessment of indoor air in Austrian apartments with and without visible mold growth. *Atmospheric Environment* 2007; 41: 5192-5201.
52. Paternoster L, Savenije OEM, Heron J*, et al.* Identification of atopic dermatitis subgroups in children from 2 longitudinal birth cohorts. *J Allergy Clin Immunol* 2018; 141: 964-971.
53. Savenije OE, Granell R, Caudri D*, et al.* Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. *J Allergy Clin Immunol* 2011; 127: 1505-1512 e1514.
54. Neuman A, Hohmann C, Orsini N*, et al.* Maternal smoking in pregnancy and asthma in preschool children: a pooled analysis of eight birth cohorts. *Am J Respir Crit Care Med* 2012; 186: 1037-1043.
55. Tischer C, Casas L, Wouters IM*, et al.* Early exposure to bio-contaminants and asthma up to 10 years of age: results of the HITEA study. *Eur Respir J* 2015; 45: 328-337.
56. Fukao T, Fukutomi O, Hirayama K*, et al.* Questionnaire-based Study on the Relationship between Pet-keeping and Allergic Diseases in Young Children in Japan. *Allergology International* 2005; 54: 521-526.
57. Liccardi G, Salzillo A, Calzetta L*, et al.* Can the presence of cat/dog at home be considered the only criterion of exposure to cat/dog allergens? A likely underestimated bias in clinical practice and in large epidemiological studies. *Eur Ann Allergy Clin Immunol* 2016; 48: 61-64.

FIGURE LEGENDS

Figure 1. Longitudinal patterns of pet and dampness or mold exposure

Figure 2. Adjusted associations of longitudinal patterns of pet and dampness or mold exposure with asthma at age 17 (N=1747) and with sensitization at age 16 (N=637) ‡

‡: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mold exposure in the home at 1 year and analyses of dampness or mold exposure were adjusted for furry pets in the home at 1 year.